The effects of Ischemia reperfusion injury on cilia length and calcium signaling.

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Introduction: Primary cilia are small microtubule-based appendages present on nearly every cell type in mammals and are important during development and throughout adulthood. Ischemia-reperfusion injury (IRI) was demonstrated to affect the length of cilia in the renal tubule. One of the signaling mechanisms thought to play an important role in renal cilia is Ca^{2+} signaling.

Methods: We propose that following IRI, additionally to the change in cilia length we will have a change on ciliary calcium signaling. To visualize cilia in live animals we use mice expressing an mCherry-tagged 5-HT6 receptor which localizes to cilia fused with a G-GECO Ca^{2+} reporter. Changes in ciliary Ca^{2+} are measured by changes in the ratio of GFP to mCherry. We performed IRI surgery on these mice and measured ciliary length and Ca^{2+} signaling at 7,14,21,35 days post-IRI. The resulting videos were analyzed using NIS-Elements software to measure cilia length and GFP:mCherry ratio.

Results: Our images show that the cilia are shorter on days 14 and 28 post IRI when compared to controls (no-IRI). When we look at days 21 and 35 post-IRI, we see increasing in cilia length compared to controls. When we compare the calcium changes after IRI we found a lower number of calcium spikes in the days 7 and 21 post-IRI compared with the days 14, 28 and 35 post-IRI.

Future directions: Based on that our next step will be using the GCaMP6f mouse which expresses a fluorescent calcium indicator protein, GCaMP6f, in the cytoplasm to see if the injury also affects cytoplasmic calcium levels or if this is unique to ciliary Ca2+.

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